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Exploring The Role Of Fungal Endophytes For The Production Of Anti-Cancerous Compound Podophyllotoxin From Podophyllum Hexandrum

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Abstract:

In an effort to discover new therapeutic compounds, research on the secondary metabolites of endophytic microbes colonising plant bodies is currently focusing on the medicinal qualities of those products. A non-alkaloid lignin of plant origin, podophyllotoxin is present in various species of Podophyllum as well as several other genera, including Juniperus, Sinopodophyllum, Dysosma, Linum, and others. A few endophytic fungi that generate podophyllotoxin as a secondary metabolite have been studied and documented in the literature that is now available. Specific endophytic fungal strains can be isolated and their metabolites extracted using conventional methods. Nowadays by utilising industrial fermentation technologies for growing fungal endophytes there is a trend to produce secondary metabolites to meet industrial demands. The present study aimed to optimise the culture conditions for isolated endophytes for the rhizosphere and phyllosphere of Podophyllum hexandrum plants grown in the in the green house of JUIT and some plants purchased from Jagatsukh nursery of Himalayan Forest Research Institute (HFRI). Phytochemical screening was carried out in mother plant extracts and isolated fungal endophytes extracts and we found significant amounts of phenols, alkaloids, glycoside and flavonoids. Experiments are under process for the enrichment of podophyllotoxin production. So the present study would provide enriched microbial resources for podophyllotoxin production which will contribute to anticancer drug development.

Keywords: ***Endophytes, Podophyllotoxin, Secondary metabolites.***

Introduction

Podophyllotoxin, a lignan derived from Podophyllum species, is commonly extracted from the rhizomes of Sinopodophyllum hexandrum and Podophyllum peltatum, which are native to Western China and the Himalayan region. Extracts of podophyllotoxin have been found to be effective in treating conditions such as syphilis, psoriasis, gonorrhea, tuberculosis, and venereal warts. Additionally, the podophyllotoxin family exhibits various therapeutic properties, including immunosuppressive, neurotoxic, antibacterial, and anti-inflammatory effects. The demand for podophyllotoxin has risen due to the challenges in cultivating P. hexandrum, which has limited availability. Endophytic fungi, known to inhabit the healthy tissues of numerous plant species, have emerged as a potential source of valuable phytochemicals, offering an alternative method of obtaining podophyllotoxin. These fungi are known to produce compounds such as flavonoids, terpenoids, alkaloids, and

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phenols, which may hold promise for developing effective therapeutic agents.[1]
In traditional medical systems like Ayurveda, Unani, and Siddha, the podophyllum plant has considerable promise for treating a wide range of maladies. Because podophyllum is neither cultivated or extensively collected, there is a shortage of podophyllotoxin, which has increased demand for the material on the global market. Because organic synthesis is not feasible, plant rhizomes of P. peltatum and P. hexandrum are used to isolate podophyllotxin. Due to a combination of aggressive harvesting and a lack of culture, P. hexandrum rhizomes—which have a dry weight basis potential of 5% podophyllotoxin—are becoming more and more difficult to find. Endophytes are often part of the microbial colony that resides inside of a plant's healthy tissues [3]. Most plant species, both dicotyledonous and monocotyledonous, include microbial endophytes. Endophytic fungus have been shown to be a varied source of phytochemical components with a broad spectrum of biological activity. Numerous experts have found that a vast array of medicinal plants naturally harbor over a million species of endophytic fungi. Flavonoids, terpenoids, alkaloids, phenols, tannins, steroids, amines, organic acids, myoinositol, volatile ester, spectrum of fatty acids, etc. are abundant in endophytic fungus. The cost and difficulty of screening biological products has increased, and the majority of endophytes contain special compounds that are going extinct faster. Numerous techniques are being employed to find new and effective antibacterial compounds. Research organizations are investing funds.

Methodology: [2]

Collection of plant materials

The leaves of podophyllum hexandrum were collected from Green House of JUIT.

Isolation of Endophytic Fungi

The gathered leaves were surface sterilized, and the isolation process was carried out with minor adjustments in accordance with Petrini's (1986) standard protocol.

Surface Sterilization

Once the samples were rinsed, they were surface sterilized in 70% ethanol for 1 minute, in 3% sodium hypochlorite for 4 minutes, and then in 70% ethanol again for thirty seconds. After that, they were given three 60-second showers using double-distilled water.

Incubation

Following sterilization, the leaves were cut into 5 mm by 2 mm pieces, which were then aseptically deposited onto Petriplates that were filled with Potato Dextrose Agar (PDA) media. The PDA was treated with 100 µg/ml of tetracycline to stop the growth of bacteria. Next, the plates were incubated for five to seven days at room temperature. After counting the number of distinct fungal colonies found on the PDA plates, each colony was separated and subcultured on a different PDA plate to produce pure cultures.

Identification of endophytic fungi

Identification of the endophytic fungus was done by observation of morphological and cultural traits. Endophytic fungi have been recognized macroscopically through the use of culture features such as pigmentation, surface roughness, colony morphology, and mycelium development on PDA. Using LPCB dye, a section of the mycelium was teased and examined under a 45X magnification for the microscopic examination of endophytic fungi. The fungus species has been identified based on the morphology of the hyphae, spores, and fruiting bodies such as conidia, mycelia growth, and conidiophores.

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Inoculation of endophytic fungi

By inoculating fungal mycelia and spores in 250 ml sterile flasks containing PDB and incubating for seven days while shaking them at 200 rpm, a fungal spore suspension was created.

Extraction of crude extract

The culture was then given 100 milliliters of ethyl acetate following the incubation period, and it was kept at room temperature for the entire night. Using a Buchner funnel, the unrefined ethyl acetate fungus combination was filtered through muslin fabric.
To get crude fungal extract, the collected supernatant was dried under vacuum on a rotary evaporator at 40 C.

Phytochemical analysis: [4]
Alkaloids

One milliliter of crude extract and two milliliters of HCl were mixed in a test tube, which was then submerged in water. Mayer's reagent (0.1 ml) was then added. There are alkaloids present when a yellow precipitate appears.

Terpinoids

After taking one milliliter of fungal extract, a few drops of sulfuric acid solution were added. Terpenoids are indicated by the formation of a reddish-brown precipitate.

Flavonoids

A few drops of diluted sodium hydroxide were added to 1 milliliter of crude extract. This produced a bright tint that eventually became colorless when a few drops of HCl were added.

Quinones

When 1 ml of 10% sodium hydroxide was added to 0.5 ml of crude extract, the mixture became blue-green or red, signifying the presence of quinones.

Phenols

A solution of 0.5 ml FeCl and 0.5 ml crude extract was mixed together. The development of phenols is $_2$ indicated by the presence of a reddish tint.

Findings:

During these studies we isolated fungal specie, which were identified using fluorescent microscopy LPCB (lacto phenol cotton blue) stain.

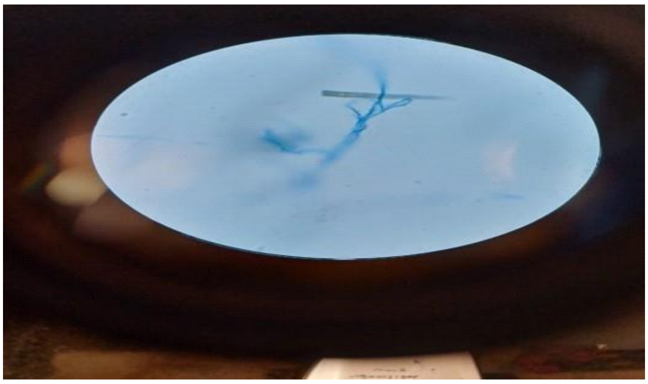

Figure no. 1: microscopic view of endophytic fungus

Screening of phytochemicals

The phytochemical analysis was carried out using ethyl acetate crude extracts of endophytic fungi. In thisprocess, phenols, terpenoids, alkaloids, flavonoids were screened

Secondary metabolites	Presence and absence
Terpenoids	+
Alkaloids	-
Flavanoids	+
Quinines	-
Phenols	+

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Qualitative screening is done till now, we are yet to perform molecular analysis of these metabolites which shows prominent hints for the availability of podophyllotoxin.

Conclusion:

In conclusion, we found that endophytes can be a potential alternative for the production of podophyllotoxin. This research not only highlights the importance of fungal endophytes as promising sources of bioactive compounds but also suggests avenues for further exploration in harnessing their biotechnological potential. As we continue to unravel the complexities of plant-fungal interactions, the future holds promise for developing sustainable and innovative approaches in drug discovery and production.

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